J. Mar. Biol. Ass. India, **51** (2) : 130 - 136, July - December 2009

†Breeding and larviculture of the sapphire devil damselfish *Chrysiptera cyanea*

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Abstract

The sapphire devil damselfish, *Chrysiptera cyanea* is one among the top ten species of marine ornamental fishes in the international trade. For the first time, broodstock development, breeding and larviculture techniques of *C. cyanea* were developed and standardised and the details are presented. Broodstock (length of fish:5 to 6.5 cm) was developed in two tonne capacity FRP tanks with biological filter and by feeding with natural feeds *ad libitum*. The number of eggs per spawning ranged from 2000 - 2500. The interval between successive spawnings ranged from 5 to 20 days. The eggs were oval - shaped and measured around 1.3mm in length and 0.6mm in width. Parental care by the male was noticed. Hatching occurred on the night of the third day of incubation. The larvae were altritial type but with mouth opening at the time of hatching. The length of newly hatched larvae averaged to 2.5mm and the mouth gape around 150µ. Larviculture was done in five tonne capacity FRP tanks by employing greenwater produced by the microalga *Nannochloropsis* sp. Different larviculture systems were experimented by varying the cell counts of greenwater and live feeds. The cell counts of greenwater employed for the experiments were in three ranges - 1 x 10^4 to 9 x 10^4 ml^{-1}, 1 x 10^5 to 9 x 10^5 ml^{-1} and 1 x 10^6 to 9 x 10^6 ml^{-1}. Four sets of experiments were conducted by feeding with different live feeds – one set with enriched rotifer (*Brachionus rotundiformis*) alone, the second set by employing mixed culture of two copepods species *viz*. *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus*, the third set by employing copepods and rotifers together as live feed and the fourth set with copepods as starter feed for the first six days followed by enriched rotifers from 7 to 15 days post-hatch (dph). The larval survival was recorded on 15th dph. Feeding experiments with *B. rotundiformis* alone and those with *B.rotundiformis* and copepods together as live feeds were not successful. Co-culturing of the two selected species of copepods in optimum range of cell count of greenwater gave the best survival. In this set, survival rate of larvae on 15 dph ranged from 5 to 8%. The maximum survival rate was 5-6% in the group fed with copepods as starter feed upto 6 dph followed by enriched rotifers from 7 to15 dph. It was noted that a cell count range of 1 x 10^5 cells ml^{-1} was the optimum, which yielded the maximum larval survival in both these sets of experiments. After 15 dph, the larvae were fed with freshly hatched *Artemia* nauplii and no further mortality was noted. Metamorphosis of larvae started from 24th day and all the larvae metamorphosed by 30th day. The technique developed has the potential to scale up to commercial level production.

Keywords: Damselfish, *Chrysiptera cyanea*, breeding, larviculture, copepods

Introduction

Hatchery production is the best environmentally sound way to increase the supply of marine ornamentals, reduce pressure on wild population and produce juveniles and marketable fish year round. In addition, hatchery produced fish are hardier and survive longer in captivity.

Species belonging to the family Pomacentridae such as clownfishes and damselfishes dominate the international trade of marine ornamental fishes

†Presented in the International Symposium "Marine Ecosystem-Challenges and Opportunities (MECOS 09)" organized by the Marine Biological Association of India during February 9-12, 2009 at Kochi.

accounting for 43% of total fish traded (Collette *et al*., 2003). The damsel fish family consists of about 235 speceis worldwide (Allen, 1991). The pomacentrids *viz., Chromis viridis, Amphiprion ocellaris, A. percula, Dascyllus aruanus, D. trimaculatus* and *Chrysiptera cyanea* are the species with high demand in the international trade. Considering this, method for breeding and seed production of the sapphire devil damselfish *Chrysiptera cyanea* was developed, several successful trials of seed production were carried out and standardised paving the way for commercial level production.

Material and Methods

***Broodstock development*:** Broodstock development was done in rectangular FRP tank (capacity: 2 t) with light green colour. The tank was fitted with biological filter to maintain water quality to the optimum level. The filtration rate was about 200 litres per hour. Six juvenile fishes of the sapphire devil *Chrysiptera cyanea* were collected by liftnet and introduced into the tank for broodstock development. The ranges of water quality parameters in the broodstock tank were as follows : temperature: 25º to 30ºC, pH : 8.3-8.6, salinity : 28-35 ppt and dissolved oxygen : 4.5-5.0 ml/litre. Water in the broodstock tank was changed at the rate of 30% once in a week. The tank was kept under translucent roofing in order to provide light. Fishes were fed once in a day *ad libitum*. Various types of feeds like finely chopped fishes, shrimps, polychaete worms and chopped clam meat were given to the broodstock. Substrata were provided in the broodstock tanks for attachment of eggs.

***Live feed culture*:** Microalgae were culture in order to develop greenwater in the larval rearing tanks and copepod and rotifer cultures were maintained for feeding the larvae during the initial phase. Pure cultures of microalga *Nanochloropsis* sp. were maintained in indoor culture rooms by employing standard methods. These cultures were then scaled up in outdoor algal production facility to the required volume. The rotifers employed for the experiments were enriched with commercial enrichment medium Algamac 2000 (Biomarine Inc., Aquafauna, USA).

Hatching and larval rearing: The substratum with egg clutch was transferred to the larval rearing tanks containing seawater having the same physicochemical characteristics of the parent tank on the evening of the day of hatching. A gentle air flow was created over the eggs by placing an air stone near the egg clutch followed by overnight incubation in darkness. Generally hatching took place on the night of 3rd day of incubation. In some cases, the eggs were hatched in the broodstock tank and the newly hatched larvae were introduced into larviculture tanks. Larval rearing was carried out in 5 tonne FRP tanks. The inner side of the tank was light blue in colour in order to have a better contrast between the live feed and the surroundings. The range of water quality parameters in the larviculture system were: temperature 27º - 31.5º C, pH 7.5 - 8.5, salinity 28 - 35 ppt and dissolved oxygen 4.5 - 5.0 ml/litre.

Greenwater technique using the microalga *Nanochloropsis* sp. was adopted for larviculture. The adults of two species of copepods *viz*., *Euterpina acutifrons* and *Pseudodiaptomus serricaudatus* were inoculated at a density of 400 – 500 numbers per litre into the greenwater. When the copepods entered growth phase, as was noted by counting the number of egg-bearing copepods and nauplii per 50 ml, about 700 newly hatched fish larvae were introduced into these tanks. Different larviculture systems were experimented at different cell counts of greenwater and larval survival was noted on day 15 of post-hatch (dph). One set of experiment was conducted by employing rotifers cultured in microalgae as live feed, the second set by employing copepods alone as live feed, the third set by employing copepods and rotifers cultured in microalgae, together as live feed and the fourth set with copepods as the starter feed for the first six days and then changing over to rotifers enriched with Algamac 2000, until 15 dph. The range of values given under each set is based on the results of three trials conducted.

Results

***Broodstock development and spawning*:** First spawning was obtained in captivity after 8 months of maintenance in the broodstock tank. The mature fish ranged in total length from 5 - 6.5 cm. One day

before spawning, the parent fishes actively cleaned the site for attaching the eggs by rubbing it with their pelvic fins and picking off any loose particles or algae with their mouths. During spawning, females attached their eggs on the cleaned site, which were immediately fertilized by the males. Spawning occurred during morning hours. Approximately 2000 - 2500 eggs were present in a single spawn. night of 3rd day at 28 - 30^0 C (Figs. 1-5). Hatching was delayed to 4th day or 5th day if the temperature range was lower. During this period the male parent took care of the eggs by protecting them and by fanning them with the pectoral fins and tail.

Larviculture: The length of newly hatched larvae averaged to 2.5 mm with an average mouth gape of 150 µ (Fig. 6). In the first set, the larvae were

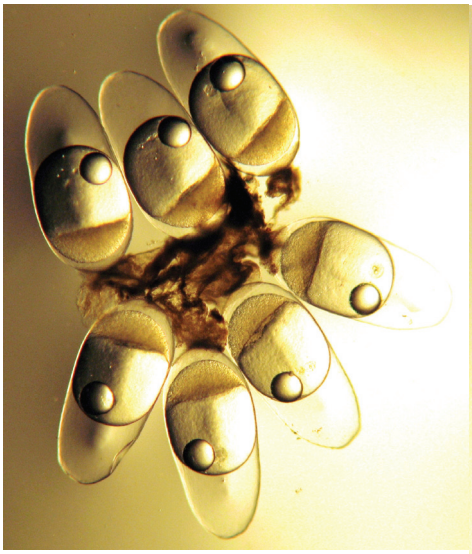

Fig. 1. A bunch of freshly laid egg of *Chrysiptera cyanea*

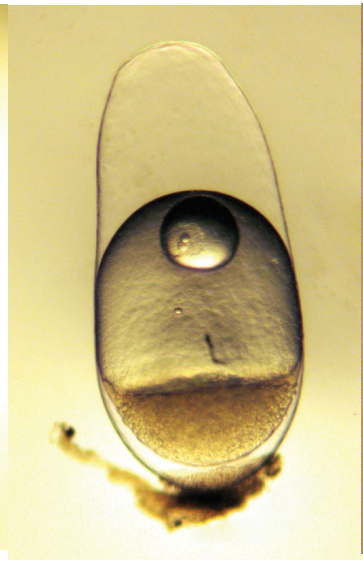

Fig. 2. Freshly laid single egg of *C. cyanea*

Fig. 3. 2nd day egg of *C. cyanea*

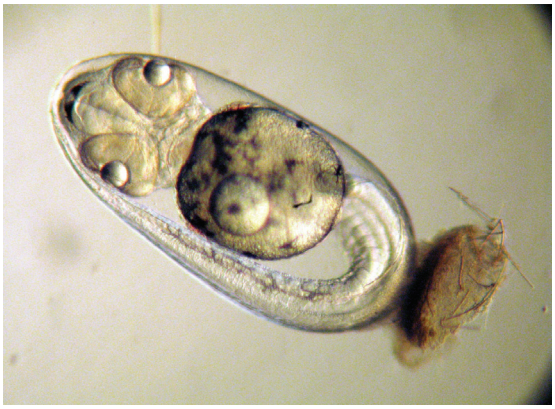

Fig. 4. 3rd day egg of *C. cyanea*

The eggs were attached on the substrata provided inside the broodstock tanks. The eggs were oval-shaped and measured around 1.3 mm in length and 0.6 mm in width. The periodicity of spawning ranged between 5 and 20 days. Hatching took place on the

Fig. 5. 4th day egg of *C. cyanea*

transferred to 5 tonne capacity FRP tanks in which greenwater of different cell counts and rotifers at an initial density of 5 to 8 numbers per ml. were maintained (Table 1). On 15 dph, no larval survival was noted.

Table 1. Larviculture systems experimented for *Chrysiptera cyanea* with rotifers as live feed

Range of cell count of green	No. of rotifers ml^{-1} (Range upto 15 dph)	Larval survival water ml^{-1} (15 dph)
1 x 10^4 to 9 x 10^4	5-12	Nil
1 x 10^5 to 9 x 10^5	5-20	Nil
1 x 10^6 to 9 x 10^6	5-22	Nil

Fig. 6. Newly hatched larva of *C. cyanea*

In the second set, the larvae were transferred to 5 tonne capacity FRP tanks in which greenwater of different cell counts and a mixed culture of copepods (*P. serricaudatus* and *E. acutifrons*) was maintained. The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval survival was noted when the cell count of the greenwater was maintained at a range of 1 x 10^5 to 9 x 10^5 cells ml^{-1} . The results of the larviculture systems experimented with copepods as live feed is given in Table 2.

The larval survival on 15 dph ranged from 5-8% in the experiments conducted with green water of cell count range 1 x 10^5 cells – 9 x 10^5 cells. After fifteen days, freshly hatched *Artemia* nauplii were supplemented. Thereafter no mortality was noted. The larvae started metamorphosing from 24th day and by 30th day all of them metamorphosed. The average length of just metamorphosed juvenile was 21 mm (Figs.7 & 8).

In the third set, the larvae were transferred to 5 tonne capacity FRP tanks in which greenwater of different cell counts and a combination of copepods and rotifers were employed as live feed (Table 3). On 15 dph no larval survival was noted.

In the fourth set, the larvae were transferred to 5 tonne capacity FRP tanks in which greenwater of different cell counts and copepods were employed as the starter live feed for 1 to 6 dph and from 7 to 15 dph with enriched rotifers (Table 4). The highest number of egg bearing copepods and nauplii in the larviculture system and the maximum larval

Table 2. Larviculture systems experimented for *Chrysiptera cyanea* with copepods as live feed

Range of cell count of greenwater ml^{-1}	Range of egg bearing copepods (Nos. ml^{-50})	Range of nauplii (Nos. ml^{-50})	Larval survival (%) on 15 dph
1 x 10^4 to 9 x 10^4	0-2	0-2	0-1
1 x 10^5 to 9 x 10^5	7-41	23-132	5-8
1 x 10^6 to 9 x 10^6	2-4	1-4	0-2

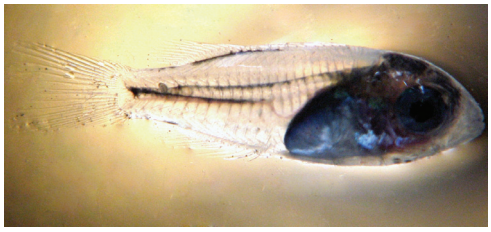

Fig. 7. *C.cyanea* larva just before metamorphosis

Fig. 8. Hatchery produced juveniles of *C. cyanea*

Table 3. Larviculture systems experimented for *Chrysiptera cyanea* with copepods and rotifers as live feed

Range of cell count of greenwater ml^{-1}	Range of egg bearing copepods (Nos. ml^{-50})	Range of nauplii (Nos. ml^{-50})	Range of rotifers (Nos. ml^{-1})	Larval survival on 15 dph
1 x 10^4 to 9 x 10^4	0-2	0-5	5-10	Nil
1 x 10^5 to 9 x 10^5	0-2	0-6	8-18	Nil
1 x 10^6 to 9 x 10^6	0-1	0-3	10-18	Nil

Table 4. Larviculture systems experimented for *Chrysiptera cyanea* with copepods as starter feed for first six days and enriched rotifers from 7th to 15th day

Range of cell count of greenwater ml^{-1}	Range of egg bearing copepods (Nos. ml^{-50}) (1-6 dph)	Range of nauplii (Nos. ml^{-50}) (1-6 dph)	Range of rotifers (Nos. ml^{-1}) (7-15dph)	Larval survival (%) on 15 dph
1 x 10^4 to 9 x 10^4	2-4	4-5	5-6	0-1
1 x 10^5 to 9 x 10^5	5-30	30-95	5-14	5-6
1 x 10^6 to 9 x 10^6	5-6	5-8	7-20	1-2

survival range of 5 to 6% was noted when the cell count of the greenwater was maintained at a range of 1 x 10^5 to 9 x 10^5 cells ml^{-1}.

Discussion

The key factors for successful larviculture of marine finfishes depend chiefly on the appropriate size and nutritional quality of live feeds. Among the marine ornamental fishes, the first success was achieved in the breeding and seed production of clownfishes, as their larviculture protocols are comparatively easy (Hoff, 1996). In India also the first success was the development of hatchery techniques of clownfishes (Gopakumar *et al*., 2001; Ignatius *et al.*, 2001; Madhu and Rema Madhu, 2002). Experimental success was also obtained in the breeding and larval rearing of damselfishes (Gopakumar *et al.*, 2002; Gopakumar *et al*., 2007). Olivetto *et al.* (2003) reported successful larval rearing of the pomacentride *Chrysiptera parasema.*

C. cyanea has altritial type of larva and the mouth gape of newly hatched larva was around 150 µ. Trials on feeding with the available strain of the rotifer *Brachionus rotundiformis* as starter feed were not successful. As conventional live feeds such as rotifer and *Artemia* could not meet larval feed requirements, attempts were focussed on the use of copepods as live feed for marine finfish larviculture. Copepods have almost become inevitable because they are the only acceptably-sized prey for small larvae of many ornamental fish species and the only type of live feed that will support the rearing of many species of marine fish which have altritial type of larvae. Copepod nauplii offer a diverse size spectra and nutrition that can meet the specialized needs of small and fast growing fish larvae.

A number of studies have shown that the inclusion of copepod nauplii into the early larval diet significantly improves the survival and growth of groupers (Hussain and Higuchi, 1980; Doi *et al.,* 1997a; Toledo *et al.,* 1999) and snappers (Singhagraiwan and Doi, 1993; Doi *et al*., 1997b; Schipp *et al.,* 1999). Larvae of *Epinephelus coioides* were shown to actively select for early stage copepod nauplii when fed with a mixture of copepod nauplii and rotifers and the larvae showed increased survival and growth when their rotifer diet was supplemented with a density of only 0.1 nauplii ml^{-1} (Toledo *et al.,* 1999). Larvae of *Lutjanus argentimaculatus* fed on rotifers and a range of copepods were shown to feed exclusively on smaller copepod nauplii from day 4 to day 6 after hatching (Doi *et al.,* 1997b). Copepods have also been employed for the larviculture of the halibut *Hippoglossus hippoglossus,* turbot *Scophthalmus maximus*, cod *Gadus morhua,* the sea bream *Archoargus*

rhomboidalis, the bay anchovy *Anchova mitchilli* and lined sole *Achirus lineatus (*Phelps, *et al.,* 2005). Experiments with the striped trumpeter *Latris lineata* larvae showed that the use of copepods improved the survival (Morehead *et al*., 2005). *Lutjanus campechanus* larvae fed with nauplii *of Parvocalanus* sp. exhibited significantly greater survival to day 7 after hatching (50.3%) and were larger in size (Shields *et al*., 2005). The small calanoid copepods *Bestiolina similis* and *Parvocalanus crassirostris* were compared with *Acartia sinjiensis* as feed for snappers and groupers (McKinnon *et al.,* 2003). It was found on the basis of size of developmental stages, susceptibility to predation, growth rate and nutritional composition that *B. similis* was the best candidate for larval fish diets.

Survival of sea bass larvae fed with *Acartia clausi* was the highest (58.13%) against 39.93% and 41.62% in larvae fed with rotifer and *Artemia* nauplii respectively (Rajkumar and Kumaraguru, 2006). A higher survival rate and better growth performance of juveniles of the long snout seahorse *Hippocampus reidi* fed on a combined diet with the harpacticoid copepod *Tisbe* spp. was recorded (Olivetto *et al.,* 2008). A positive effect of the supplementary feeding with *Tisbe* spp. as well as with the calanoid copepod *Centropages typicus* was noted in the clownfish *Amphiprion clarkii* larvae (Olivetto *et al.,* 2008). Two species of copepods *viz., Euterpina acutifrons,* a harpacticoid copepod and *Pseudodiaptomus serricaudatus,* a calanoid copepod were cultured and employed as starter feed for the larviculture of three species of damselfishes *viz.,* the threespot damselfish, *Dascyllus trimaculatus,* the humbug damselfish, *Dascyllus aruanus* and the blue damselfish, *Pomacentrus caeruleus*. It was found that co-culturing of copepods in greenwater in the larviculture tank is the most effective method for the species studied (Gopakumar and Santhosi, 2007; Gopakumar *et al.,* 2007).

In the present study, co-culturing of the selected two species of copepods *viz., P. serricaudatus* and *E. acutifrons* in greenwater along with larvae yielded positive results. The small size of the first naupliar stages of the copepods and the availability of different sizes of nauplii during the initial phase of larviculture had initiated and sustained the first exogenous feeding of the larvae. The initial stages of nauplii noted in the larviculture system measured from 60 to 80 µ, which was suitable for the first feeding of the larvae. The high EPA, DHA and ARA content of copepods also would have facilitated larval survival and growth.

The maintenance of copepods in multiplicative phase in the larviculture system is the crucial factor for the survival of the larvae. The cell count range of 1 x 10^4 to 9 x 10^4 cells ml^{-1} would have been too low for the multiplication of the copepods. The cell count range of 1 x 10^6 to 9 x 10^6 appears to be too high as it would have affected the filter feeding of the copepods. Hence the cell count range 1 x 10^5 to 9 x 10^5 cells ml^{-1} appears to be optimum for multiplication as was indicated by the maximum number of egg bearing copepods and nauplii. The naupliar count alone cannot be taken as an indicator of multiplication due to the fact that most of the newly hatched nauplii will be consumed by the larvae. The better survival of the larvae can be directly attributed to the availability of freshly hatched nauplii which were indicated by the abundance of egg bearing copepods and nauplii in the larviculture system. It is felt that survival rates could be further enhanced if the copepods in the larviculture system could be kept at optimum production level.

The larviculture systems experimented with copepods as starter feed during 1-6 dph and then to enriched rotifers during 7–15 dph also gave comparable results to those fed entirely with copepods from 1–15 dph. This indicates that the first feeding of the larvae during the initiation of exogenous feeding is met by the copepod naupliar stages. After 6 dph, enriched rotifers can be substituted for copepods.

The larviculture systems experimented with copepods and rotifers together as live feeds were not successful. The rotifers multiplied rapidly by parthenogenesis and occupied the system. The copepods, being sexually reproducing, could not keep pace with rotifer multiplication and were rapidly eliminated from the system. The larvae of *C. cyanea* were unable to accept rotifers as starter feed which resulted in total mortality of the larvae.

It is also noted that the critical phase of larviculture was over by 15 dph. After 15 dph, the mouth gape reached around 450 µ and can be fed with freshly hatched *Artemia* nauplii. The absence of any mortality from this stage onwards indicated that once the starter feed problem is solved, the larviculture could be accomplished easily with conventional live feeds.

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Received : 12/02/2009
Accepted : 10/07/2009